



DNA Markers for Genetic Identification of Species – Provenance – Individuals

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● Avoid problems due to changes in fishing effort

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Discrimination Power / Utility Summary

(Applications for Fisheries Management)

<u>Technique</u>	<u>Species ID</u>	<u>Provenance</u>	<u>Individual</u>
Protein Gels	Y	?	-
mtDNA	Y	Y	-
DNA Microsats	-	-	Y
SNPs	Y	Y	Y

CSIRO – Oceans & Atmosphere - Australia

(Fishery Independent Data - Based on Genomics)

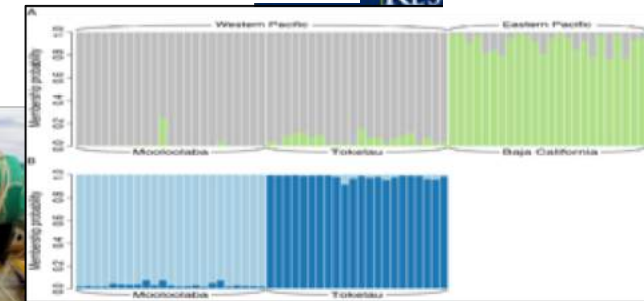
- Abundance and fishery monitoring
- Stock Structure/Provenance, chain of custody



Discrete pan-Pacific yellowfin populations

Western Pacific

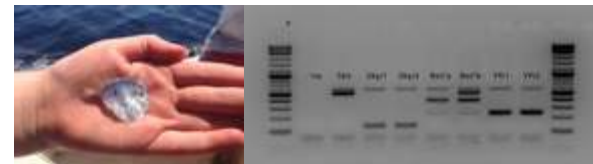
Eastern Pacific



- Gene Tagging



- Species ID



P.M. Grewe, et al., *Evidence of discrete yellowfin tuna (Thunnus albacares) populations demands rethink of management for this globally important resource*, *Marine Freshwater Research* 67(8):1081-1089

Bradford et al., 2016
Scientific Reports 5: 16916 (2015)

CSIRO Oceans & Atmosphere – Strategic Research

(Fishery Independent Data - Species ID/Stock Structure/Gene Tagging)

Initial Approach

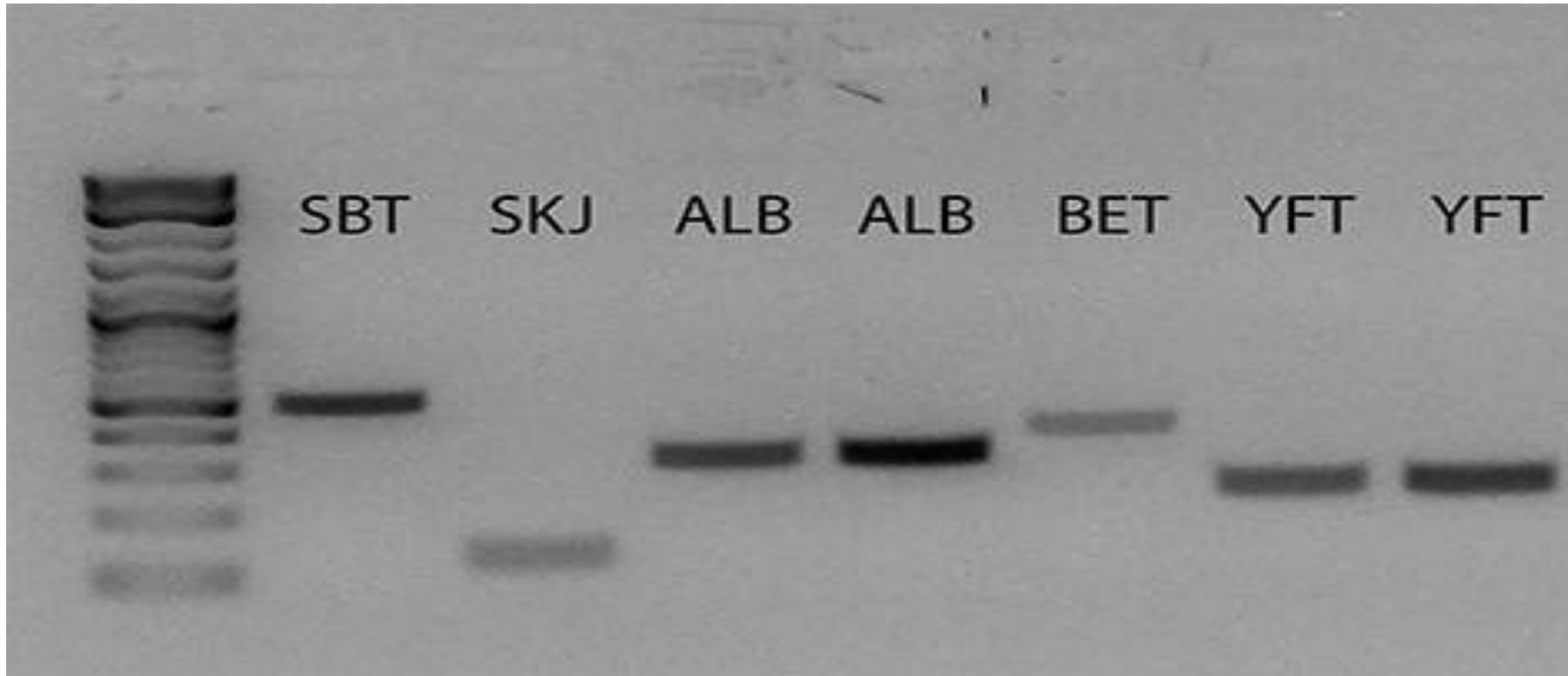
- CSIRO O&A used internal strategic funds to develop baseline genetics (DNA markers, assay techniques, statistical approaches, pop'n modelling)

Extending Work - External Funding/Collaborations (Tuna, Neritics, Billfish, Sharks)

- Atlantic (**NOAA, VIMS, AZTI**)
(USA, Spain)
- Indian Ocean (**RITF, RIMF, MRC, AZTI, IRD, IFREMER, ACIAR, MSC, DFAT, IOTC/FAO**),
(Indonesia, Maldives, Solomon Islands, Spain, France, PNG, others....)
- Pacific Ocean (**FRDC, SPC, ICAAT, NOAA, WCPFC**)
- **CCSBT**, projects looking at CloseKin/Mark-Recapture & GeneTag



Species ID Using mtDNA Markers



Practical application :

- Tools to detect species substitution promoting Truth in labeling
- Verification of Catch documentation to identify IUU
- Check your own samples for sneakers (mislabeling accidents)

Single Nucleotide Polymorphism – SNPs

- **Genotyping by Sequencing – complexity reduction**
(several techniques demonstrated power of these approaches)

- **Provides species level discrimination**

(Davies et al., 2016 - Report to TRAFFIC and CCSBT Secretariat, 2 October 2016)

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Tracing Individuals Through the Supply Chain

City	Shanghai	Shanghai	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing	Beijing
Source	Canada	Canada	Kagoshima, Japan	Japan	Indonesia	Japan	Japan	Spain, from agent	Nagasaki, Japan	Atlantic	Nagasaki, Japan	Japan/Canada	Japan	Spain
Species ID	ABT	ABT	NBT	ABT	NBT	NBT	NBT	NET	NET	ABT	ABT	ABT	ABT	ABT
Date sampled	7 Jan	7 Jan	19 Jan	19 Jan	19 Jan	15 Feb	19 Feb	19 Feb	20 Feb	16 Mar	16 Mar	14 Mar	15 Mar	17 Mar
Sample ID	21	21	58	58	74	93	93	94	95	100	167	173	183	194
Colour code	Bluefin Tuna	Bluefin Tuna	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper	Yellowtail Snapper
Explanation	1st observation of individual fish	1st observation of duplicate individual	2nd observation of duplicate individual	3rd observation of duplicate individual										

olve population structure (prove
T, ALB, SKP, ABT)

nt for Individual identification (Po
se-kin mark-recapture and gene-
identification through DNA profiling, CSI-Miami etc..

- Can resolve population structure (provenance)

(e.g. YFT, BET, ALB, SKP, ABT)

- **Excellent for Individual identification (Po'P, FSP, HSP)**

(e.g. close-kin mark-recapture and gene-tagging)

(Individual identification through DNA profiling, CSI-Miami etc....)

Individual Identification via Gene Tagging

(Funded by CCSBT as part of ongoing recruitment monitoring)

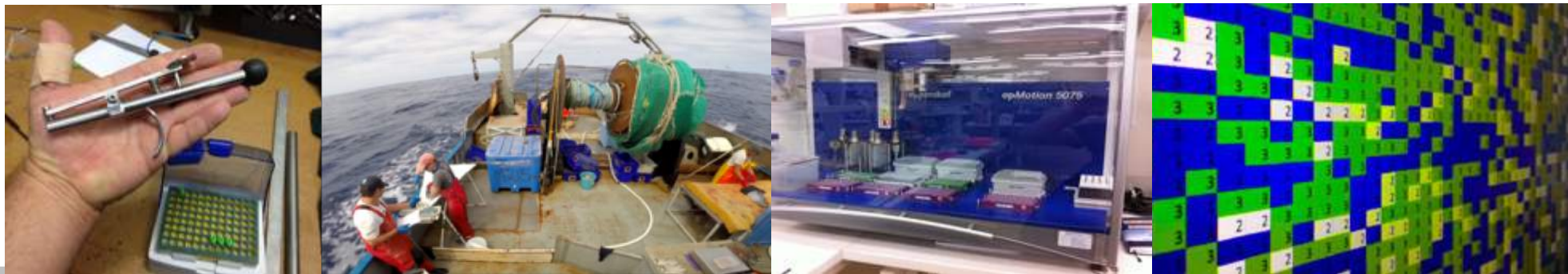
- **Individual Identification**

Southern bluefin tuna (SBT) using 60 markers)

- **Mark Recapture** – Absolute abundance estimates of recruitment for Stock Assessment and Management Procedures.

(~20,000 fish per year, sampling 6/minute)

- **Product chain of custody**

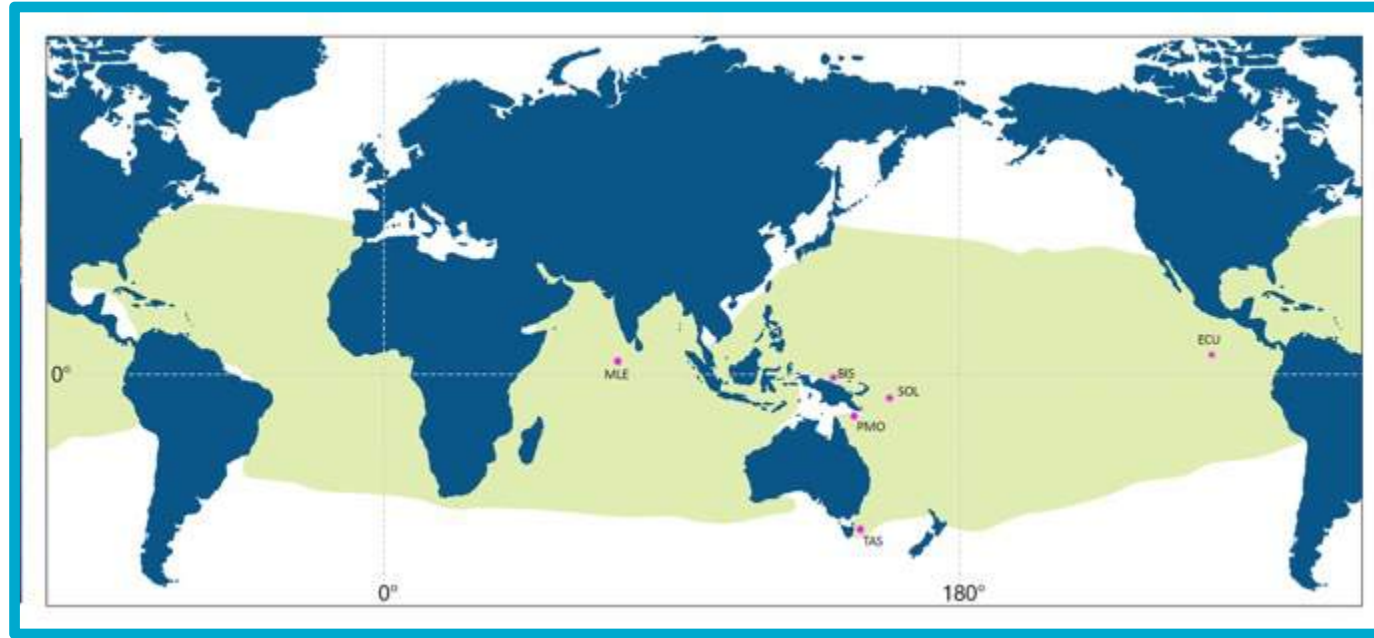


CSIRO – Stock Structure Program – Collaborators

(Fishery Independent Data Based on Genomics)

- CSIRO development of baseline genetics & tissue collection (20+ years)
(strategic research internally funded as well as co-investment with partners)
- Australian Centre for International Agricultural Research (**ACIAR**), **BET / YFT**
(Indonesia, Maldives, Solomon Islands)
- Marine Stewardship Council (**MSC**), **Skipjack**
(Maldives, Indonesia, Papua New Guinea)
- Inter-American Tropical Tuna Commission (**IATTC**), **BET, YFT, SKJ**
- Indian Ocean Tuna Commission (**IOTC**), **Neritics / Tropical Tuna / Sharks**
(Maldives, Indonesia, France, Spain, others....)
- Australian Government Fisheries Research & Development Council (**FRDC**)
Albacore / Bigeye / Yellowfin / Striped Marlin / Swordfish
- **WCPFC** tissue bank and people at **SPC**, **ALB/BET/YFT/....etc...**

Skipjack – Structure (MSC & IATTC)

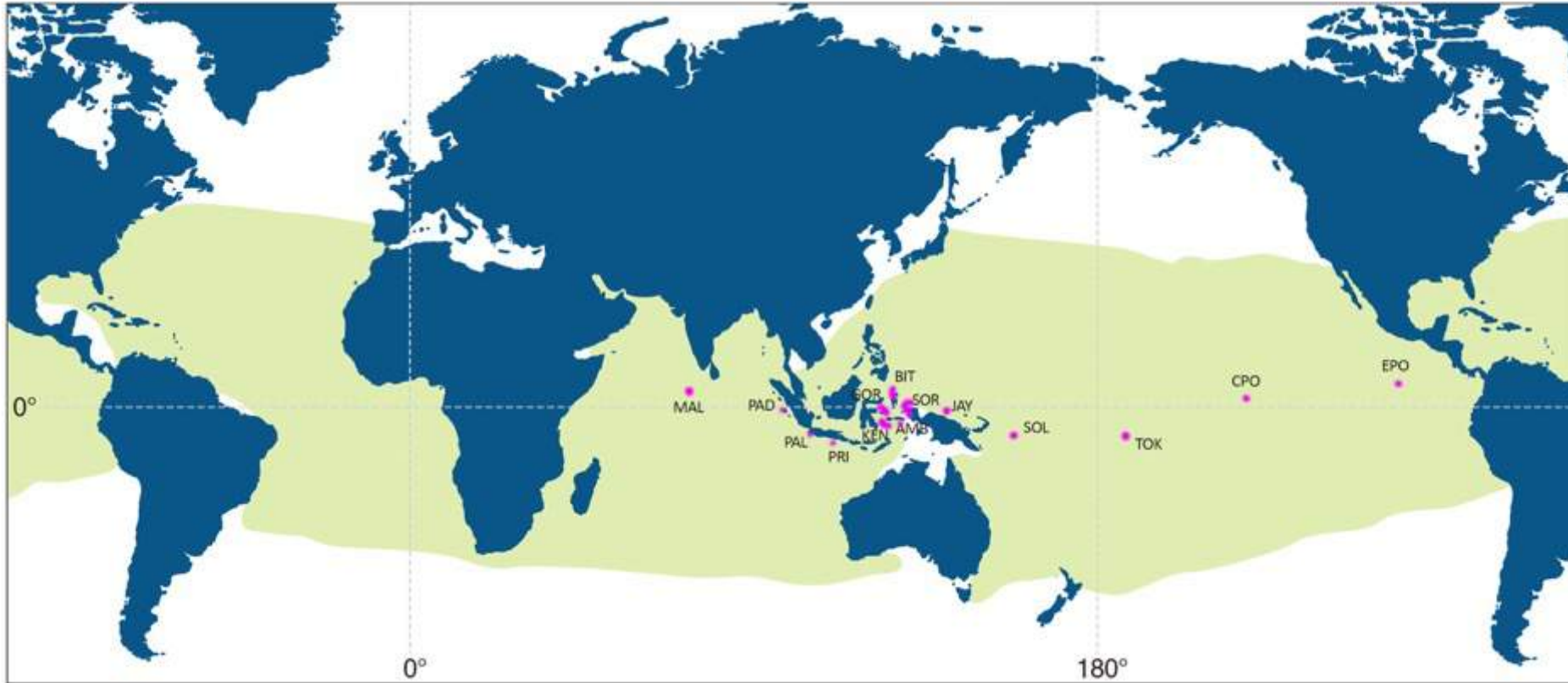


Maldives, Bismark Sea, Port Moresby, Solomons, Tasmania, Ecuador

- Provenance of individuals is very difficult for skipjack Tuna.
- Maldives and Ecuador genetically different ($p < 0.001$).

BET – Sampling Locations

(CSIRO, ACIAR, Indonesia, IATTC)



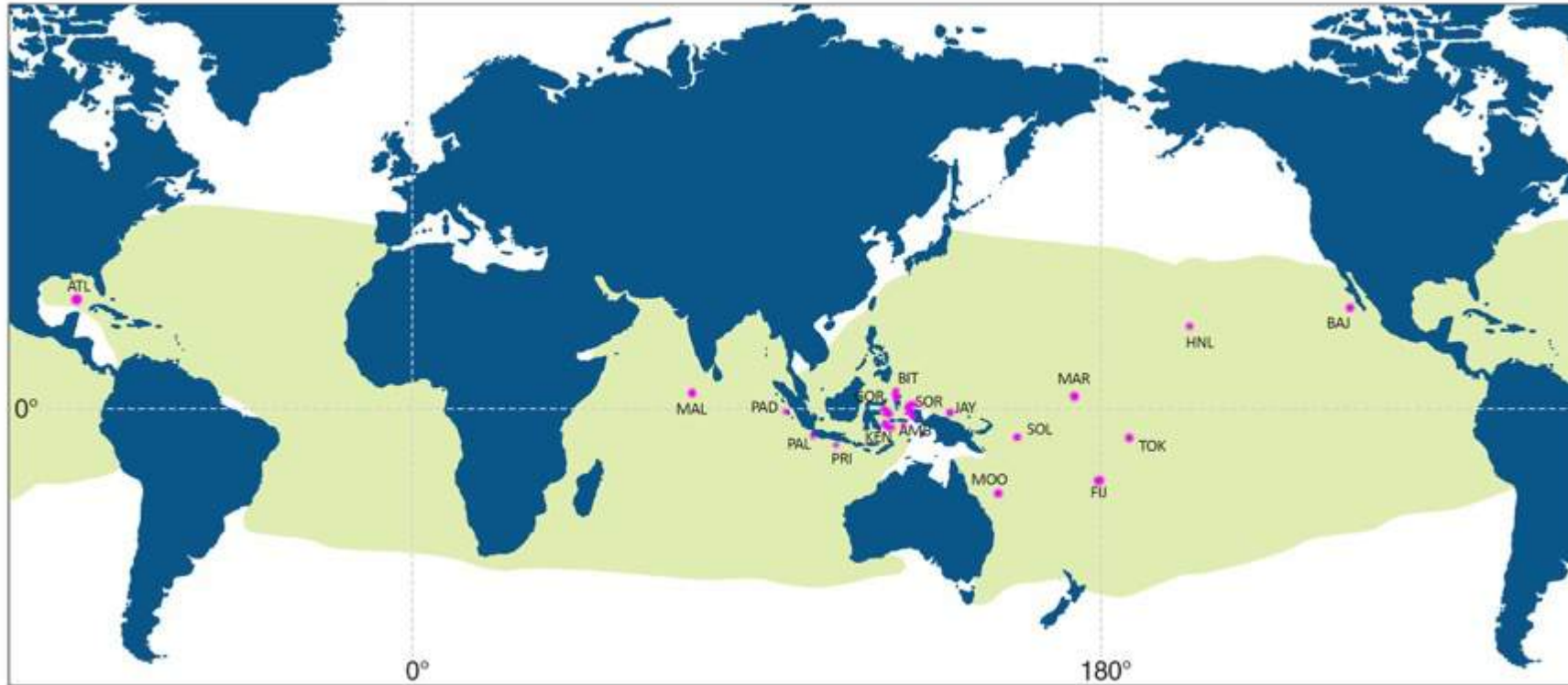
Maldives, Indonesia (9 sites), WPO, CPO, EPO

BET – Genetic Analysis

- Differentiation of fish present at an Ocean basin scale (Indian and Pacific Oceans).
- Differences appeared to be temporally stable
- In the Pacific there was significant differentiation between the WPO & EPO ($p < 0.001$)

YFT – Distribution & Sampling

(CSIRO, ACIAR, IATTC, NOAA)

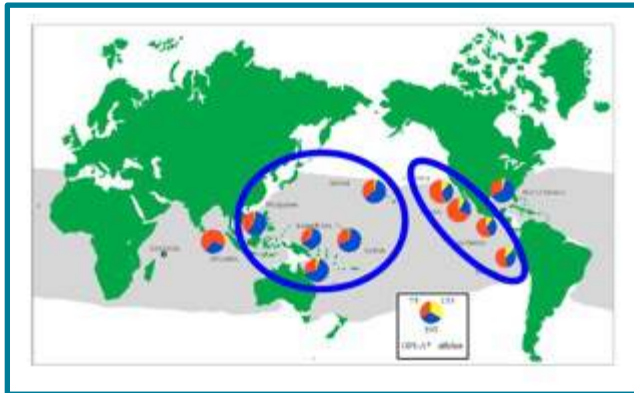


Gulf of Mexico, Maldives, Indonesia, WPO, CPO, EPO

YFT – Genetic Analysis

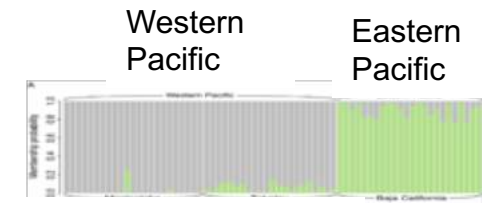
- Differentiation of fish at an Ocean basin scale (signal was temporally stable)

Proteins



DNA SNPs

Heterogeneity across Pacific Ocean yellowfin, (left panel) using allozymes and using SNPs (right panel)



- DNA markers could identify provenance of individuals of western versus eastern Pacific Ocean

Successful Application of Genetic Tools

(Lessons for Genomics Based Fishery Management)

- **Requires effective integration of DNA approaches & broad scale genetic coverage / marker validation**
- **Collaboration with management agencies and member countries responsible for managing pelagic species.**
- **Identify important questions relevant to management, compliance, and supply chain.**
- **Establish a good research plan/sampling strategy.**

Summary Outcomes & Future Directions

- **Identified DNA markers for determination of Species-ID, Provenance, & Individuals
(*e.g.* Bigeye, Skipjack, Southern Bluefin, Yellowfin)**
- **Good spatial and temporal coverage, but, continue sampling to fill gaps in species distribution**
- **Complete development of a rapid test to deliver a low cost, high throughput, forensic grade test**

Discussion & Questions

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